

DEVELOPMENT OF EDIBLE/BIODEGRADABLE PACKAGING BASED ON κ -CARRAGEENAN WITH SPENT COFFEE GROUNDS AS ACTIVE ADDITIVES*

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Edible packaging with active and intelligent properties has gained recent attention, although the concept of edible packaging itself is not new. These packages are created using various substances such as polysaccharides, proteins, and lipids, either individually or in combination. To find the optimal combination for specific applications, the physical and chemical properties of these packages are measured. Active or intelligent properties are achieved by incorporating substances, often extracts, with antioxidant, antibacterial, or other beneficial properties into the packaging. The aim of the research was to develop an edible packaging based on κ -carrageenan, with spent coffee grounds as potential additives to impart active properties of edible/biodegradable packaging. To assess the suitability of the packaging for future applications, the thesis evaluated its textural properties, including thickness, water content, flexibility, and strength. Additionally, the antioxidant activity and free radical scavenging capacity of the packaging were measured using various methods. Elasticity of the packaging was increased ($p < 0.05$) with the addition of spent coffee grounds, the same as polyphenolic compounds ($p < 0.05$). While recent attempts have been made to utilize spent coffee grounds for various purposes, their incorporation into edible/biodegradable packaging remains certainly not enough explored. The research emphasized the possibility of the incorporation of spent coffee grounds into edible/biodegradable matrices and the findings will serve as a good foundation for further studies.

Keywords: antioxidant activity, textural properties, free radical scavenging capacity, polyphenolic compounds

Introduction

Ranked second only to oil, coffee stands as a global commodity of immense trade significance, reflecting its universal popularity and economic impact. Beyond its trade value, coffee's cultural diversity and environmental challenges underscore its role as a complex and influential global commodity [1].

The literature substantiates the staggering fact that the yearly consumption of coffee beans registers at an astounding magnitude, hovering around an estimated 8 million tons. This substantial figure underscores the profound relationship between coffee and daily life, as billions of individuals around the world partake in this cherished beverage, contributing not only to its economic significance but also to its cultural ubiquity [2].

Spent coffee grounds (SCG), notable for their elevated moisture content and substantial organic load, present a distinct advantage owing to their convenient availability for collection from a range of sources including cafeterias, restaurants, and factories. The dual attributes

of high humidity and organic content inherent to SCG not only enhance its feasibility for collection but also render it an ideal substrate conducive to fostering mold activity, thereby further highlighting its potential for various applications [3].

SCG exhibits a distinct compositional profile, characterized by the virtual absence of reducing sugars as it consists mainly of hemicellulose (30–40 wt.%), lignin (20–30 wt.%), cellulose (8–15 wt.%), proteins (13–17 wt.%), lipids (7–21 wt.%) and ash (1–2 wt.%). According to its composition, SCG can be used for polysaccharide extraction, polymer precursors, biopolymer production, and as a fermentation substrate [4]. The oil fraction, which can be present up to 20%, can be used in the production of biodiesel and bioethanol [5,6]. Additionally, one of the most promising uses of SCG is based on its high antioxidative potential. An essential contributor to the antioxidant potency of SCG is the presence of polyphenolic compounds. Particularly noteworthy is the

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of chlorogenic acid and its derivatives and derivatives: caffeoylquinic, feruloylquinic and p-coumaroylquinic acids, and mixed diesters of caffeic, ferulic and quinic acid [7]. These components are the principal phenolic constituents within SCG, constituting as much as 14% of its dry matter content. This unique composition not only offers insights into SCG's antioxidant potential but also accentuates its role as a valuable resource [7-9].

The primary aim of the study was to investigate the feasibility and potential benefits of incorporating spent coffee grounds (SCG) into edible packaging materials. Through an analysis of SCG's compositional attributes, such as its polyphenolic content and antioxidant activity, this research seeks to assess how SCG can contribute to enhancing the functional and sustainable properties of edible packaging. By exploring the interaction between SCG and packaging materials, this study aims to provide valuable insights into the development of eco-friendly packaging solutions while concurrently valorizing SCG as a bioresource.

Material and methods

Preparation of edible packaging with the addition of spent coffee grounds

Preparation of the control sample: 0.3 g of κ-carrageenan (Merck Life Science spol. s r.o., the Czech Republic) was mixed in 45 ml of distilled water so that there were no lumps in the mixture and it was homogeneous. The mixture was heated with constant stirring for 15 minutes at about 50 °C. 0.25 mL of glycerol (PENTA s.r.o., the Czech Republic) was added to the heated mixture and allowed to heat for an additional 5 minutes. Then the mixture was poured into a Petri dish (9 cm diameter).

Production of packaging with the addition of spent coffee ground (100 % Arabica): Samples of four different concentrations of spent coffee grounds were experimentally produced. The preparation procedure was the same as for the control sample, 0.3 g of κ carrageenan was weighed and then spent coffee grounds were added. The amount of coffee grounds depended on the concentration, for a concentration of 0.5%: 0.225 g was weighed; for 1%: 0.45 g was weighed; for 1.5%: 0.675 g; and for 2%: 0.9 g of spent coffee grounds. Then 45 ml of distilled water was added, the mixture was thoroughly mixed and heated for 15 minutes at approx. 50 °C with constant stirring. 1.5 ml of glycerol was added to the mixture, which was mixed for 5 minutes with constant heating. The mixture was then poured into a Petri dish.

Table 1. The composition of experimentally produced edible packaging

Samples	Ingredients
Control	0.3 g κ- carrageenan + distilled H ₂ O + glycerol
KL 0.5%	0.3 g κ- carrageenan + distilled H ₂ O + 0.225 g spent coffee grounds + glycerol
KL 1%	0.3 g κ- carrageenan + distilled H ₂ O + 0.45 g spent coffee grounds + glycerol
KL 1.5%	0.3 g κ- carrageenan + distilled H ₂ O + 0.675 g spent coffee grounds + glycerol
KL 2%	0.3 g κ- carrageenan + distilled H ₂ O + 0.9 g spent coffee grounds + glycerol

All samples, including the control one, were left to dry for 48 hours. An overview of the composition of edible packaging is listed in Table 1.

Thickness

The thickness of the edible packaging was measured using a Mitutoyo M310-25 micrometer (Kawasaki, Japan) at five different locations on each sample, and the average thickness was calculated from the measured data.

Water content and solubility

The edible packaging samples were cut in squares (2 × 2 cm) and weighed (W1 value), then placed in an Eco-cell 55 oven (BMT Medical Technology s.r.o., the Czech Republic) (105 °C) for 2 h and then weighed again (W2 value). These squares were then placed in beakers with 25 ml of distilled water, after 24 hours at room temperature they were completely dissolved.

$$\text{Water content (\%)} = [(W1 - W2) / W1] \times 100$$

Textural properties

Five rectangles (5 × 1 cm) were cut from each type of edible packaging, and these were measured with a TA.XT plus texturometer (Godalming, UK) for strength

(MPa) and elasticity (%). The measurements were made according to ASTM international test methods - ATSM D882-02.

Preparation of extracts for chemical analyses

From each experimentally produced edible packaging 0.1 g was weighed and then mixed with 20 ml of a mixture consisting of 96% ethanol and distilled water in a ratio of 1:1.

Determination of total polyphenols content

The total content of polyphenols was determined by the Folin-Ciocalteu method [10]. The sample (1 g) was weighed and 10 ml of distilled water was added to it, the mixture was left in a shaker for 10 minutes, and filtered. A blank sample was prepared by adding 1 ml of distilled water to a 25 ml volumetric flask, to which 5 ml of Folin-Ciocalteu solution (PENTA s.r.o., the Czech Republic) (diluted 1:10 with distilled water) and 4 ml of Na₂CO₃ (PENTA s.r.o., the Czech Republic) (75 g/l) were subsequently added. The solution was then incubated in the dark for 30 minutes. Before the measurement, the solution was supplemented with distilled water to the mark. In a 25 ml volumetric flask, 1 ml of the extract was mixed

with 5 ml of Folin-Ciocalteu solution (diluted 1:10 with distilled water) and 4 ml of Na_2CO_3 (75 g/l). The mixture was incubated for 30 minutes without access to light. Before the measurement, the mixture was topped up to the mark with distilled water. The same procedure was repeated with all extracts. The absorbance was measured at a wavelength of 765 nm against a blank sample. The results were calculated in mg gallic acid/g.

Antioxidant capacity measured by FRAP (ferric reducing antioxidant power) method

The ferric reducing antioxidant power (FRAP) method was used based on Behbahani et al. [11]. The blank sample was prepared in a dark bottle, 960 μl of distilled water and 7.2 ml of working solution (50 ml of acetate buffer + 5 ml of TPTZ solution + 5 ml of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution) were added, and the solution was incubated for 8 minutes in the dark. A blank sample was used to reset the spectrophotometer to a wavelength of 593 nm. The solution was extracted in sealed vials without the access of light for 30 minutes in an ultrasonic bath. From these extracts, the samples were prepared in dark bottles, to which 180 μl of the prepared extract, 300 μl of distilled water and 3.6 ml of the working solution were added. All solutions were incubated for 8 min in the absence of light. The absorbance at a wavelength of 593 nm was measured. The results were expressed in $\mu\text{g/ml}$ of Trolox, which was used as a standard.

Determination of antioxidant activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method

This method for assessing the antiradical (antioxidant) activity of edible packaging was used based on Adilah et al. [12]. It consists in the reaction of the tested substance with the stable radical DPPH \cdot (2,2-diphenyl-1-picrylhydrazyl). The presence of antioxidants with antiradical activity causes the reduction of colored ($\lambda_{\text{max}} = 517 \text{ nm}$) DPPH to a colorless neutral liquid. The procedure was that 1 g of the homogenized film was weighed and 20 ml of 96% ethanol was added to it. The extract was extracted in an ultrasonic bath for 30 minutes, then filtered. 3 mL of the freshly prepared extract was taken, to which 1 mL of 0.1 mM ethanolic DPPH solution (0.0039 g DPPH (Merck Life Science spol. s r.o., the Czech Republic) in 96% ethanol) was added. A reference solution (diluted DPPH solution) was prepared in a test tube, 3 ml of 96% ethanol was mixed with 1 ml of 0.1 mM DPPH. The prepared solutions were shaken and then incubated in the dark for 30 minutes. Absorbance at 517 nm was measured for all samples including the diluted DPPH solution.

Determination of the antioxidant activity with the ABTS (TEAC method), 2,2' azinobis(3 ethyl 2,3 dihydrobenzothiazole 6 sulfonate)

This method is used to determine the total antioxidant activity, which was used based on Dordevic et al. [13]. It tests the ability of the sample or substances to quench

the radical ABTS $\cdot+$ (2,2' azinobis (3 ethyl 2,3 dihydrobenzothiazole 6 sulfonate)). Procedure: the reaction solution was prepared by mixing 10 mL of ABTS solution (0.0384 g of ABTS (Merck Life Science spol. s r.o., the Czech Republic) dissolved in a 10 mL volumetric flask in distilled water) with 10 mL of potassium peroxodisulfate (Merck Life Science spol. s r.o., the Czech Republic) solution (0.0662 g dissolved in a 100 mL volumetric flask in distilled water) and has been left in the dark at room temperature. Extracts from samples of edible packaging were prepared: 0.1 g of the sample was mixed with 20 ml of 96% ethanol in a small dark bottle, and then extraction was performed using ultrasound for 30 minutes, and the samples were then filtered. By mixing 96% ethanol with distilled water in a 1:1 ratio, 40 μl of the reference sample was prepared. Before use, the reaction solution was diluted to have a final absorbance of 0.7, and then 2.5 mL of 96% ethanol was added. Absorbance was measured at 735 nm.

Antioxidant activity measured by the CUPRAC method (Cupric ion reducing antioxidant capacity)

The CUPRAC method is based on the reduction of copper cation Cu^{2+} to copper cation Cu^+ by antioxidants in the sample. The sample (0.1 g) was weighed into a dark bottle and 20 ml of solvent (96% ethanol and distilled water in a ratio of 1:1) was added. The prepared extract was left in an ultrasonic bath for 30 minutes, and filtered. The blank sample was prepared in a 10 ml test tube. 2 ml of Cooper (II), 2 ml of Neocuproin, 2 ml of buffer and 2.2 ml of solvent (96% ethanol: $\text{H}_2\text{O} = 1:1$) were pipetted into it. The blank was incubated in the dark for 1 hour. The actual sample was prepared in a test tube with a volume of 10 ml. 1 ml of Cooper (II), 1 ml of Neocuproin, 1 ml of buffer, 0.1 ml of solvent (96% ethanol: $\text{H}_2\text{O} = 1:1$) and 1 ml of extract were pipetted into it. The sample was incubated in the dark for 1 hour. The samples were then measured using a spectrophotometer [14]

Statistical analysis

A one-factor ANOVA test, conducted by the IBM SPSS software, was used for statistical evaluation of the results. Levene's test showed parametric and non-parametric results. In the first case, the Turkey test was used, and in the second case, the Games-Howel test was used to detect statistically significant differences ($p < 0.05$) between the analyzed samples.

Results and discussion

Table 2 is summarizing the results of measuring the thickness of packages, water content, strength and braking strain of experimentally produced packaging with the addition of spent coffee ground. As the amount of added SGC increased, the thickness increased too. The thickness of the control samples versus the other samples had a statistically significant difference ($p < 0.05$). Typically, edible/biodegradable packaging are crafted within the range

Table 2. Thickness, water content, strength and braking strain of packaging with the addition of spent coffee grounds

Samples	Thickness (mm)	Water content (g)	Strength (MPa)	Braking strain (%)
Control	0.08±0.01 ^A	35 ± 1.64 ^A	0.053 ± 0.021 ^{AB}	74.47 ± 1.77 ^A
KL 0.5 %	0.36 ± 0.02 ^B	23.38 ± 0.81 ^B	0.025 ± 0.004 ^A	79.96 ± 5.05 ^{AB}
KL 1 %	0.46 ± 0.04 ^C	19.38 ± 0.89 ^C	0.034 ± 0.005 ^A	82.19 ± 0.82 ^B
KL 1.5 %	0.52 ± 0.05 ^{CD}	18.40 ± 0.41 ^C	0.035 ± 0.013 ^{AB}	85.44 ± 4.78 ^B
KL 2 %	0.69 ± 0.1 ^D	15.41 ± 0.69 ^D	0.051 ± 0.008 ^B	85.83 ± 2.68 ^B

*Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

of 0.010 mm to 0.100 mm in thickness [15]

The water content of the packaging samples (Table 2) decreases with increasing SCG content. The KL 1% and KL 1.5% samples are the only ones that did not differ statistically significantly ($p > 0.05$). Similar results were found in the previous studies by Liu et al. [16] and Jancíková et al. [17]. The decrease in water content with the addition of SCG can be explained by the reactions of phenolic hydroxyl groups in coffee with hydroxyl groups in carrageenan, and these intramolecular interactions (for example, hydrogen bonds) can affect the interaction between carrageenan and water [16].

A high water content affects the durability of the manufactured package and packaged product. A low moisture content allows longer protection of the packaged product, while a high water content can facilitate microbiological damage. On the other hand, packaging with a higher moisture content is less fragile, adapts better to the shape of the food and is therefore easier to handle [18].

From the strength textural measurement results (Table 2), it can be read that the control and the KL 2% samples were the strongest. Control, sample KL 0.5% and KL 1% were not statistically significantly different ($p > 0.05$). Control sample had the highest strength.

The reduction in packaging strength can be attributed to weak interactions of phenols and their esters (from spent coffee grounds) with hydrophilic groups present in the carrageenan matrix [13]. The breaking strain increased with the amount of SCG (Table 2). No statistically significant difference was found between the control and the KL 0.5% sample ($p > 0.05$); control samples had significantly ($p < 0.05$) lower braking strain from other samples.

The addition of SCG increased the flexibility of the packaging samples. This may be explained by the formation of hydrogen bonds between polysaccharide and polyphenols in SCG [19].

Table 3. Total polyphenol content, FRAP, DPPH, ABTS and CUPRAC activities (TPC) in packaging with the addition of SCG

Samples	TPC (mg gallic acid/g)	FRAP (Trolox µg/ml)	DPPH (%)	ABTS (%)	CUPRAC (µmol Trolox/g)
Control	0.35 ± 0.04 ^A	0 ± 0 ^A	2.3 ± 0.1 ^A	0.14 ± 0.1 ^A	0.25 ± 0.04 ^A
KL 0.5 %	2.03 ± 0.09 ^B	4.67 ± 0.65 ^B	76.5 ± 0.8 ^B	2.29 ± 0.04 ^B	18.57 ± 0.26 ^B
KL 1 %	3.91 ± 0.03 ^C	12.13 ± 0.1 ^C	68.2 ± 0.3 ^C	4.26 ± 0.08 ^C	35.64 ± 0.29 ^C
KL 1.5 %	5.40 ± 0.08 ^D	16.39 ± 1.38 ^D	28.6 ± 0.4 ^D	6.07 ± 0.56 ^D	50.78 ± 1.50 ^D
KL 2 %	6.09 ± 0.47 ^E	21.65 ± 0.12 ^E	54.7 ± 0.3 ^E	7.15 ± 0.32 ^E	57.88 ± 0.68 ^E

*Values are expressed as mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

The total polyphenols content, FRAP, DPPH, ABTS and CUPRAC in the experimentally produced packaging with SCG are summarized in the Table 3. The content of measured polyphenols is increasing. There is a statistically significant difference between all samples ($p < 0.05$). Therefore, the addition of SCG had a high effect on the polyphenolic content.

In addition, the total polyphenolic content of phenols was quantified in the control samples, a measurement that correlates with the presence of sulfated groups within the carrageenan structure. The same explanations can be done for the obtained antioxidant activities measured in control samples [17, 20].

The obtained results confirmed that the addition of SCG increased the antioxidant properties of carrageen-

an films. As it could be expected, in most measurements it was found that a higher content of SCG, thus a higher content of polyphenols (TPC), caused greater antioxidant activity. But, the correlation of total polyphenol content with DPPH and FRAP measurements was only partially detected [21]. This can be explained by the fact that not every polyphenolic compound has antioxidant properties. Another explanation is that FRAP, DPPH, and CUPRAC measurements involve different conditions. The measured values of FRAP, ABTS and CUPRAC raised gradually with the increase SCG addition, meaning that the addition of SCG had a significant effect ($p < 0.05$) on the antioxidant properties.

SCG is a useful fortification compound for various edible and biodegradable matrices since it is high in bioactive components, primarily polyphenols. The development and extensive usage of plastics made from petroleum has undoubtedly provided producers and consumers with convenience, but it has also contributed to environmental problems by encouraging addiction to this type of packaging and material. The biodegradable packaging can be fortified with the inclusion of coffee waste, such as SCG, while at the same time SCG, a waste product, becomes a valuable raw material. The use of SCG in the creation of biopolymers is acknowledged in the literature [22].

Conclusion

The study's findings reveal that the inclusion of spent coffee grounds (SCG) in packaging material has a significant impact on various properties. As SCG content increases, packaging thickness, polyphenolic content, and antioxidant activity all rise. Additionally, water content decreases, impacting product protection and durability. While SCG enhances flexibility, it weakens packaging strength due to interactions with the carrageenan matrix. These results emphasize the potential of SCG as a valuable resource for bolstering both packaging attributes and sustainability efforts, aligning with the global drive towards eco-friendly alternatives in material development and waste management.

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Izvod

RAZVOJ JESTIVOG/BIORAZGRADIVOG PAKOVANJA NA BAZI κ -KARAGENANA SA TALOGOM KAFE KAO AKTIVNIM ADITIVOM

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Pakovanja koja su jestiva i poseduju aktivna i inteligentna svojstva privlače pažnju u poslednje vreme, iako sama ideja o jestivom pakovanju nije nova. Ova pakovanja se stvaraju korišćenjem različitih supstanci kao što su polisaharidi, proteini i lipidi, pojedinačno ili u kombinaciji. Kako bi se pronašla optimalna kombinacija za specifične namene, merene su fizičke i hemijske osobine ovih pakovanja. Aktivna ili inteligentna svojstva postižu se unošenjem supstanci, često ekstrakata, sa antioksidativnim, antibakterijskim ili drugim korisnim svojstvima u pakovanje. Cilj istraživanja bio je razvoj jestivog pakovanja na bazi κ -karagenana, s dodatkom iskorišćene kafe kao potencijalnog aditiva za dodavanje aktivnih svojstava jestivom/biorazgradivom pakovanju. Kako bi se procenila pogodnost pakovanja za buduće primene, teza je ocenjivala njegove teksturalne osobine, uključujući debljinu, sadržaj vode, fleksibilnost i čvrstoću. Dodatak iskorišćene kafe povećao je elastičnost pakovanja ($p < 0,05$), isto kao i polifenolni spojevi ($p < 0,05$). Iako su nedavno preduzeti pokušaji da se iskoriste iskorišćeni talog kafe u različite svrhe, njihovo uključivanje u jestiva/biorazgradiva pakovanja svakako nije dovoljno istraženo. Istraživanje je naglasilo mogućnost unošenja iskorišćene kafe u jestive/biorazgradive matrice, a rezultati će poslužiti kao dobra osnova za dalja istraživanja.

Ključne reči: antioksidativna aktivnost, osobine tekstura, kapacitet sakupljanja slobodnih radikala, polifenolna jedinjenja